

Amendments to Claims

Claims 1-38 (Canceled).

Claim 39 (Previously Amended). A method for conditionally activating a transgene in a plant comprising:

- 1) providing constructs comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element the having general structure P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG1; and
 - d) a fourth recombinase element having the general structure P4-RS2-STP-RS2-TG2;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second promoter;
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG1 is a first transgene sequence and 3' region;
- (ix) TG2 is a second transgene sequence and 3' region;
- (ix) P3 is a third promoter; and
- (x) P4 is a fourth promoter;

wherein P1, P2, P3 and P4 are operably linked to their down stream elements and wherein TG1 and TG2 are different trait transgenes and wherein P3 and P4 are activated in a second generation plant;

- 2) providing a first and second plant selected from the group consisting of:
 - a) a first plant comprising the first and third recombinase elements and a second plant comprising the second and fourth recombinase elements;

- b) a first plant comprising the first and fourth recombinase elements and a second plant comprising the second and third recombinase elements;
- 3) crossing the first and second plants to produce a first generation plant wherein conditional expression of the first recombinase coding sequence (R1) under the control of the P1 promoter in the common germline of the first generation, excises the stop fragment from the second recombinase element allowing expression of the second recombinase coding sequence and 3' region (R2) under the control of P2 promoter, which recombinase, in turn, excises the stop fragments from the third and fourth recombinase elements, permitting expression of the trait gene(s) TG1 and TG2 under the control of P3 and P4 promoter, respectively, in the second generation.

Claim 40 (Original). A method according to Claim 39 wherein, TG1 is a trait gene, TG2 is a lethal gene that blocks plant development, and the third promoter (P3) is expressed earlier in the plant life cycle than the fourth promoter (P4) in the second generation.

Claim 41 (Currently Amended). A method according to Claim 39 wherein TG1 is a trait gene, TG2 is a sterility gene that prevents pollen formation and seed set, and the third promoter (P3) is expressed earlier than P4 in the next generation.

Claim 42 (Previously Amended). A method according to either of Claims 81 or 82 wherein the first promoter is a constitutive and common germline promoter, and the second promoter is floral common germline promoter, and the third promoter is seed-specific; wherein conditional expression of the first recombinase coding sequence in common germline under the control of the first promoter results in expression of the second recombinase coding sequence under the control of the second promoter in floral common germline, and wherein expression of the second recombinase results in the expression of the trait in the progeny seed.

Claim 43 (Previously Amended). A method according to either of Claims 83 or 84 where the first promoter is inducible and responsive to an inducing agent.

Claims 44-69 (Cancelled).

Claim 70 (Previously Amended). A method for the conditional expression of a transgene in a plant comprising:

- (i) providing a multiplicity of recombinase elements, each recombinase element comprising:
 - a) at least one promoter;
 - b) a DNA fragment;

wherein the DNA fragment comprises at least one genetic element selected from the group consisting of: a recombinase coding sequence, a site-specific recombinase sequence responsive to a recombinase, a stop fragment and a transgene;

- (ii) introducing at least two of the recombinase elements of step (i) into at least one plant wherein the at least two recombinase elements are selected from the group consisting of:
 - a) a recombinase element having a first recombinase under the control of a promoter; and
 - b) a recombinase element having a second recombinase under the control of a promoter whose expression is dependent on the expression of the first recombinase;
- (iii) activating the promoter of step (ii)(a) wherein the expression of the second recombinase is effected by the expression of the first recombinase.

Claims 71-79 (Canceled).

Claim 80 (Previously Amended). A trait expression construct comprising:

- a) a first recombinase element comprising a first promoter operably linked to a sequence encoding a first recombinase;
- b) a second recombinase element comprising a second promoter, a stop fragment bounded by site specific sequences responsive to the first recombinase and a sequence encoding a second recombinase wherein the presence of the stop fragment inhibits expression of the second recombinase, and wherein the first and second recombinases are different; and
- c) a DNA molecule bounded by site specific sequences responsive to the second recombinase;

wherein expression of the first recombinase excises the stop fragment from the second recombinase element, operably linking the second promoter and the sequence encoding the second recombinase, and wherein expression of the second

recombinase results in site specific recombination within the DNA molecule bounded by site specific sequences responsive to the second recombinase.

Claim 81 (Previously Added). A method for conditionally activating a transgene in a plant comprising:

- 1) providing constructs comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second promoter;
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG is a transgene sequence and 3' region; and
- (vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, and the activation of P3, driving expression of TG, occurs concomitantly with or after P2, driving expression of R2;

- 2) providing a transgenic plant comprising the first, second and third recombinase elements;
- 3) activating P1 such that the R1 recombinase coding sequence is expressed in a first generation plant, wherein expression of R1 excises the stop fragment from the second recombinase element;
- 4) activating P2 such that R2 is expressed, wherein expression of R2 excises the stop fragment from the third recombinase element allowing

expression of the transgene in the first and all subsequent generations of plants.

Claim 82 (Currently Amended). A method for conditionally activating a transgene in a second generation plant comprising:

- 1) providing constructs comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first germline promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second floral specific promoter selected from the group consisting of: ~~floral common germline promoters, floral male germline promoters and floral female germline promoters~~;
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG is a transgene sequence and 3' region; and
- (vi) P3 is a third promoter which is not expressed in floral tissue;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, in the first generation common germline cells and the activation of P3, driving expression of TG, occurs in the second generation;

- 2) providing a transgenic plant comprising the first, second and third recombinase elements;
- 3) activating P1 such that the R1 recombinase coding sequence is expressed in the common germline of a first generation plant, wherein

- expression of R1 excises the stop fragment from the second recombinase element;
- 4) activating P2 such that R2 is expressed in the flower of the first generation plant ~~common germline of the first generation plant~~, wherein expression of R2 excises the stop fragment from the third recombinase element allowing expression of the transgene in the second and all subsequent generations of plants.

Claim 83 (Previously Added). A method for conditionally activating a transgene in a plant comprising:

- 1) providing constructs comprising:
- a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second promoter;
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG is a transgene sequence and 3' region; and
- (vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, and the activation of P3, driving expression of TG, occurs concomitantly with or after P2, driving expression of R2;

- 2) providing a transgenic plant comprising the third recombinase element;
- 3) transforming the transgenic plant of (2) with either the first recombinase element to generate a first plant or the second recombinase element to generate a second plant;

- 4) crossing the first and second plants such that expression of R1 is expressed and excises the stop fragment from the second recombinase element allowing expression of R2 under the control of P2 which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 in the first and subsequent generation(s).

Claim 84 (Currently Amended). A method for conditionally activating a transgene in a second generation plant comprising:

- 1) providing constructs comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first germline promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second floral specific promoter selected from the group consisting of: ~~floral common germline promoters, floral male germline promoters and floral female germline promoters~~;
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG is a transgene sequence and 3' region; and
- (vi) P3 is a third promoter which is not expressed in floral tissue-;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1 in the first generation common germline cells and the activation of P3, driving expression of TG, occurs in the second generation;

- 2) providing a transgenic plant comprising the third recombinase element;

- 3) transforming the transgenic plant of (2) with either the first recombinase element to generate a first plant or the second recombinase element to generate a second plant;
- 4) crossing the first and second plants such that expression of R1, under the control of P1 in the common germline of the first generation, excises the stop fragment from the second recombinase element allowing expression of R2 under the control of P2 in the flower of the first generation plant ~~common germline of the first generation plant~~ which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 in the second and subsequent generation(s).

Claim 85 (Previously Added). A method for conditionally activating a transgene in a plant comprising:

- 1) providing constructs comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second promoter;
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG is a transgene sequence and 3' region; and
- (vi) P3 is a third promoter;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1, driving expression of R1, and the activation of P3, driving expression of TG, occurs concomitantly with or after P2, driving expression of R2;

- 2) providing a transgenic plant comprising the first, second and third recombinase elements;
- 3) inducing the first promoter such that R1 is expressed under the control of P1 in the first generation, wherein R1 excises the stop fragment from the second recombinase element allowing expression of R2 under the control of P2, which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 promoter in the first and subsequent generation(s).

Claim 86 (Currently Amended). A method for conditionally activating a transgene in a second generation plant comprising:

- 1) providing constructs comprising:
 - a) a first recombinase element having the general structure P1-R1;
 - b) a second recombinase element having the general structure P2-RS1-STP-RS1-R2;
 - c) a third recombinase element having the general structure P3-RS2-STP-RS2-TG;

wherein:

- (i) P1 is a first germline promoter;
- (ii) R1 is a first recombinase coding sequence and 3' region;
- (iii) RS1 is a first recombinase site responsive to a first recombinase;
- (iv) P2 is a second floral specific promoter ~~selected from the group consisting of: floral common germline promoters, floral male germline promoters and floral female germline promoters;~~
- (v) RS2 is a second recombinase site responsive to a second recombinase;
- (vi) STP is a stop fragment;
- (vii) R2 is a second recombinase coding sequence and 3' region;
- (viii) TG is a transgene sequence and 3' region; and
- (vi) P3 is a third promoter which is not expressed in floral tissue;

wherein P1, P2 and P3 are operably linked to their down stream elements, and wherein the temporal expression specificity of each promoter is such that the activation of P2, driving expression of R2, occurs concomitantly with or after P1,

driving expression of R1, in the first generation common germline cells and the activation of P3, driving expression of TG, occurs in the second generation;

- 2) providing a transgenic plant comprising the first, second and third recombinase elements;
- 3) inducing the first promoter such that expression of R1, under the control of P1 in the common germline of the first generation, excises the stop fragment from the second recombinase element allowing expression of R2 under the control of P2 in the flower of the first generation plant ~~common germline of the first generation plant~~, which, in turn, excises the stop fragment from the third recombinase element, permitting expression of the trait gene(s) under the control of P3 promoter in the second and subsequent generation(s).